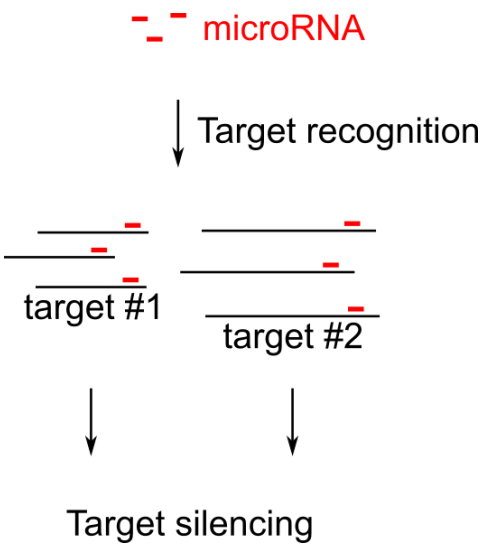
Computational Prediction of microRNA Degradation Inducers

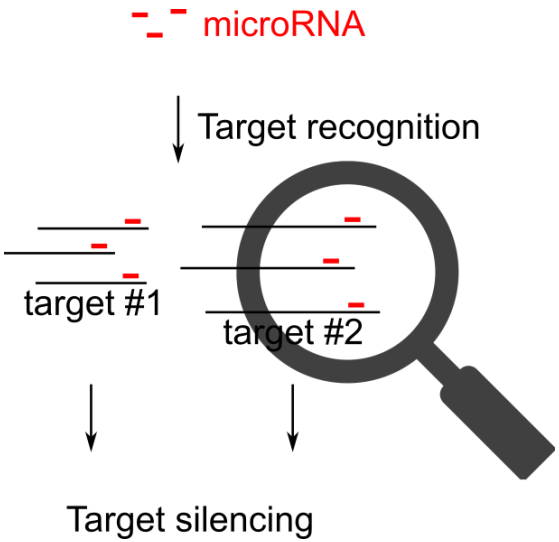
PhD Student : **Sophie Mockly**
Supervisor : **Hervé Seitz**

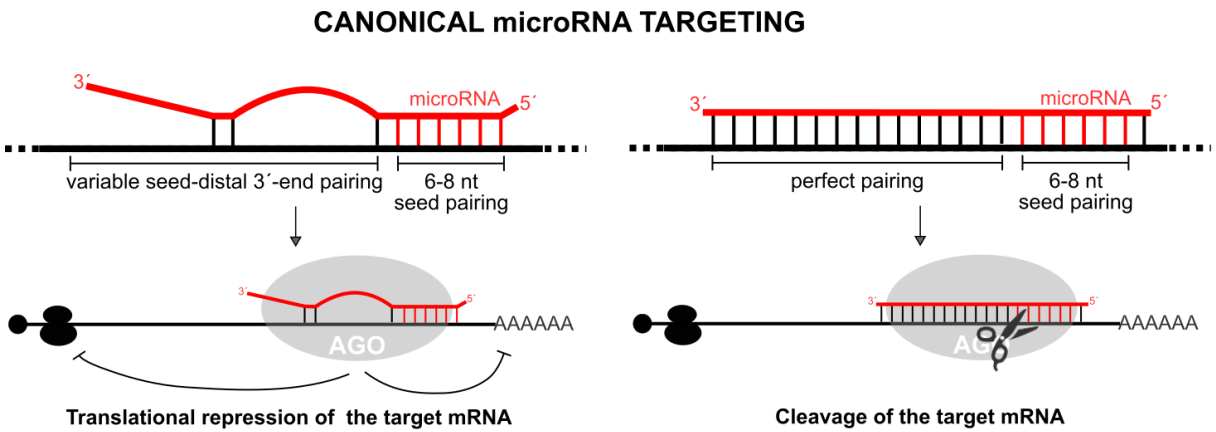
Team ``**Systemic impact of small regulatory RNAs**”
Institute of Human Genetics, Montpellier

Reciprocal interaction between miRNAs and their targets

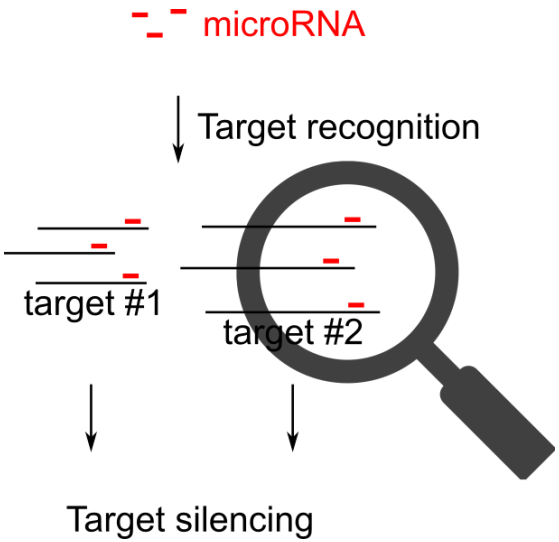


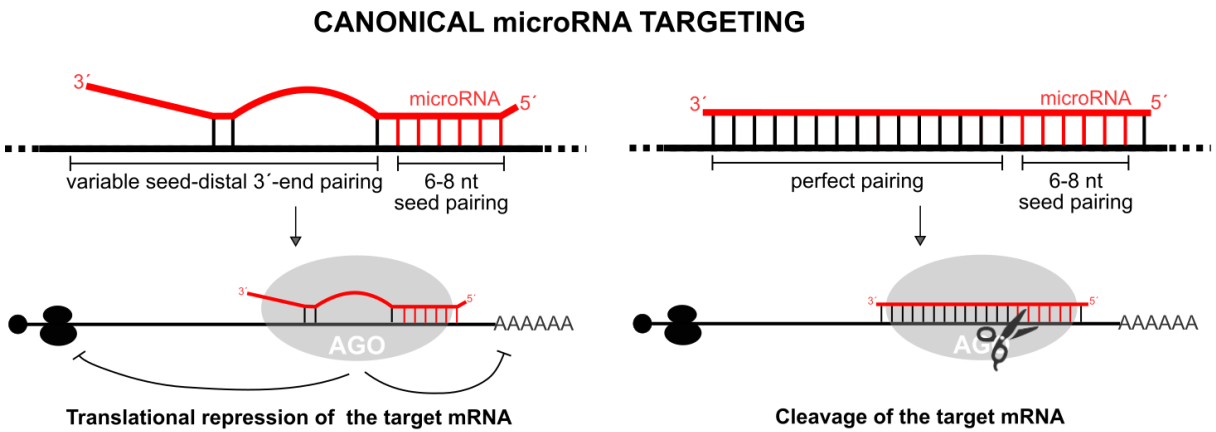
Reciprocal interaction between miRNAs and their targets

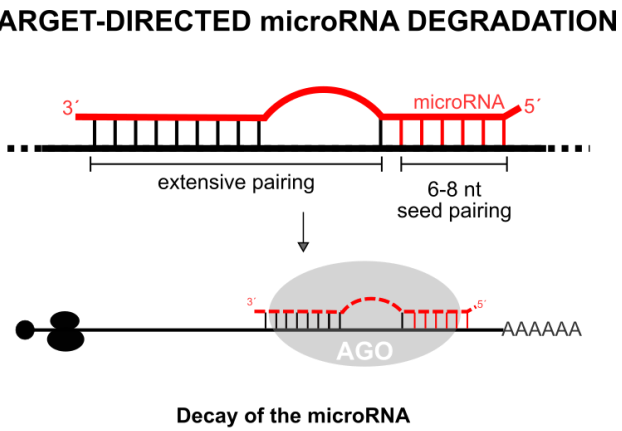




Reciprocal interaction between miRNAs and their targets

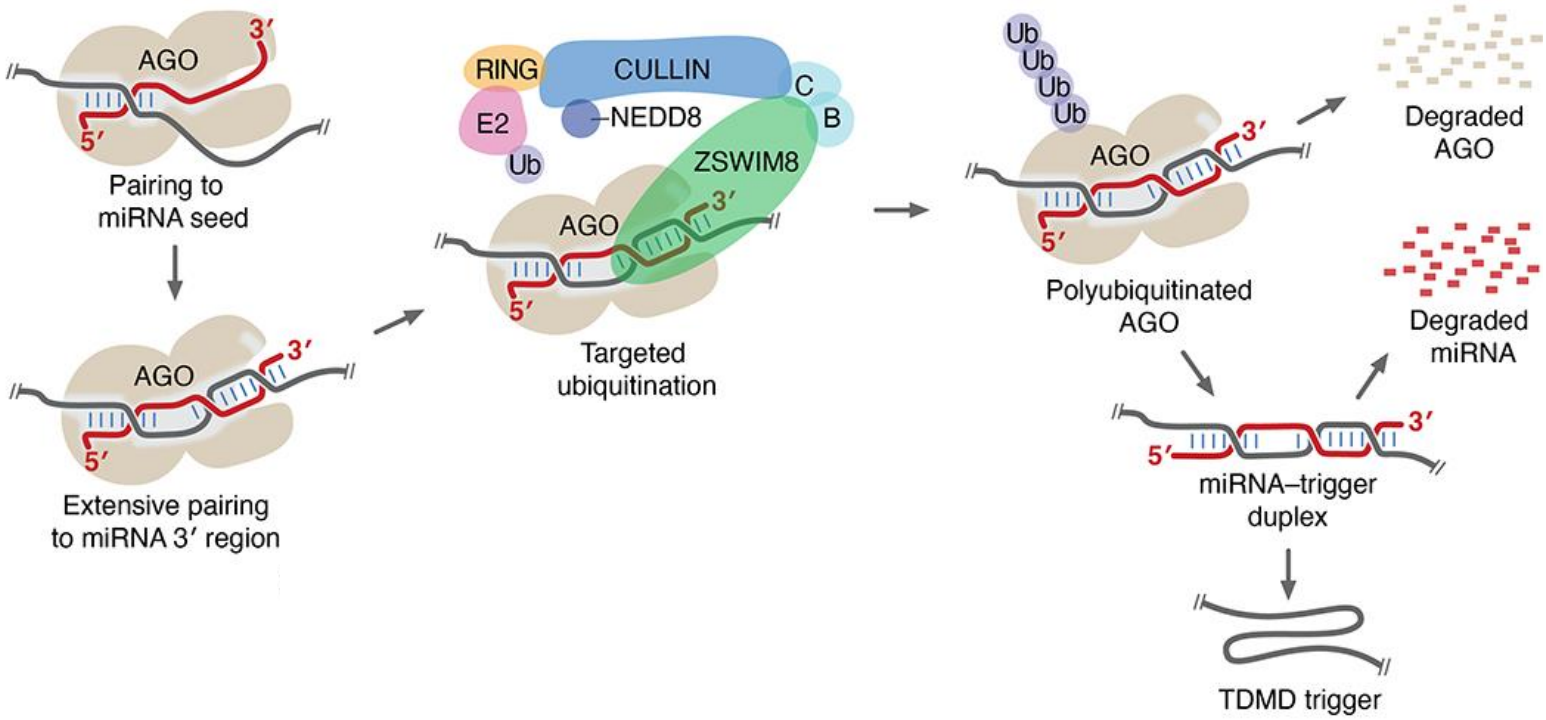






Lee et al, 2013 (Cell Host Microbe)
Bitetti et al, 2018 (Nat Struct Mol Biol)
Kleaveland et al, 2018 (Cell)

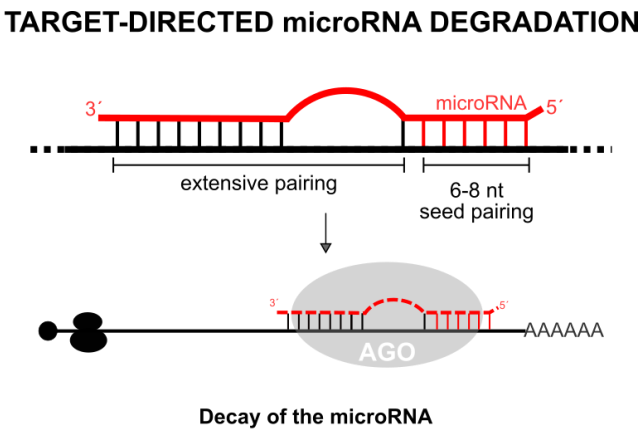
The ZSWIM8 Cullin-RING E3 ubiquitin ligase model of TDMD



Shi et al, 2020 (Science)
Han et al, 2020 (Science)

Computational Prediction of miRNA Degradation Inducers

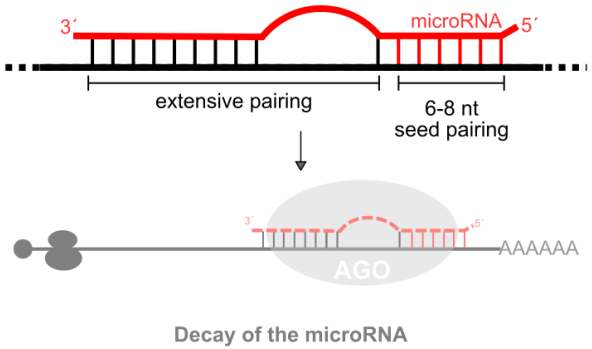
Project : **Computational identification of TDMD-inducer candidate sites**



Computational Prediction of miRNA Degradation Inducers

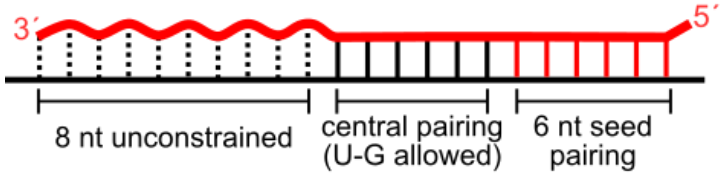
Project : **Computational identification of TDMD-inducer candidate sites**

TARGET-DIRECTED microRNA DEGRADATION

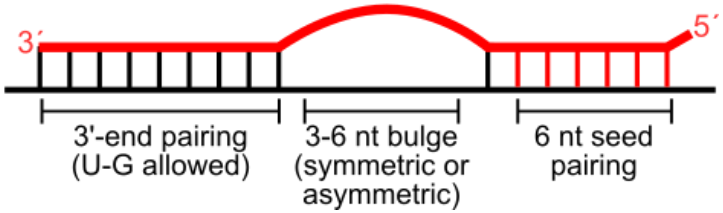


TDMD-inducing pairing geometry:

3´ mismatches:

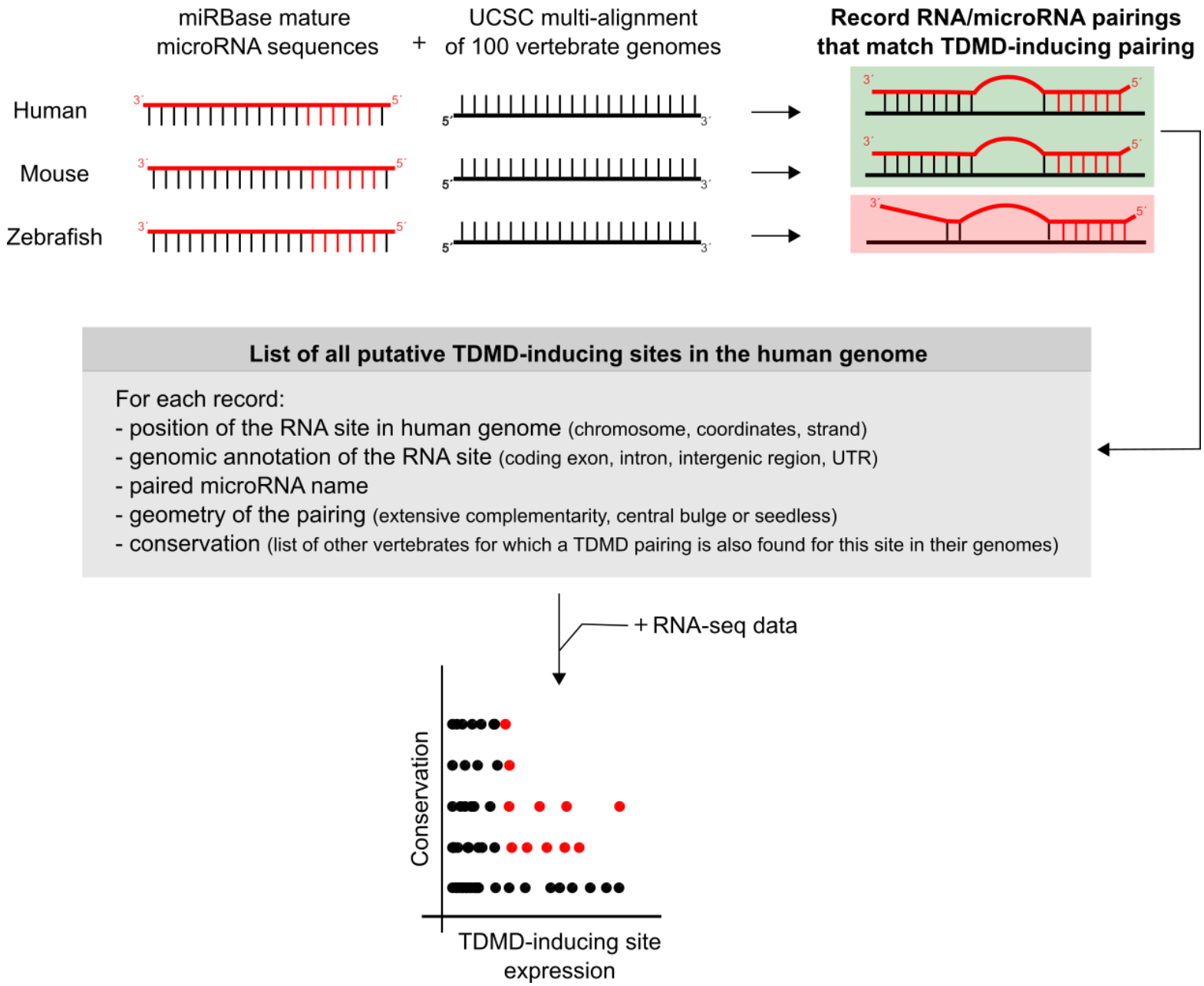


Central bulge:



From 30 examples of active TDMD inducers & 7 examples of inactive sites

Workflow overview



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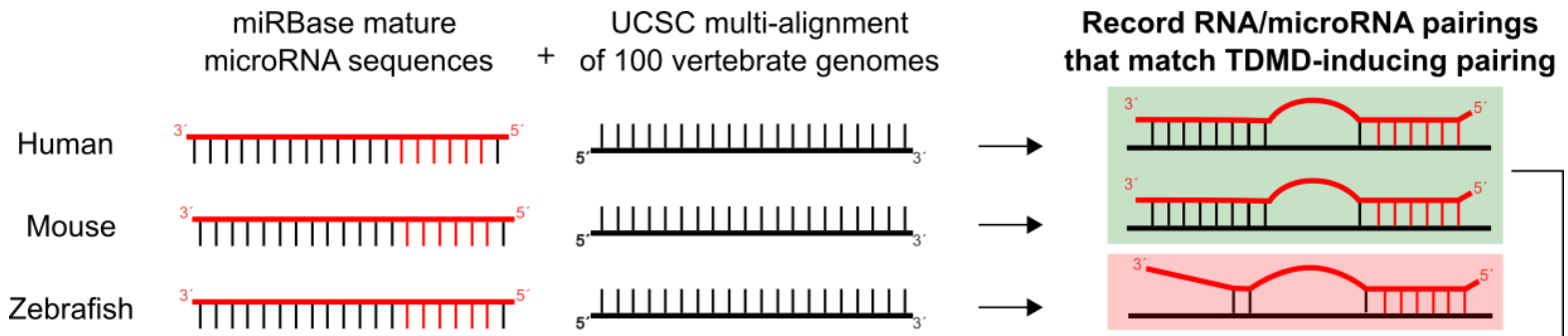
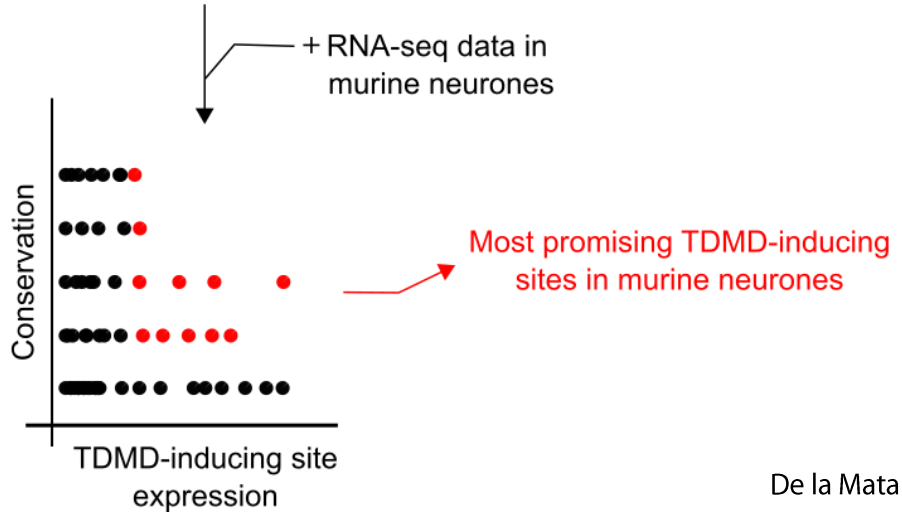


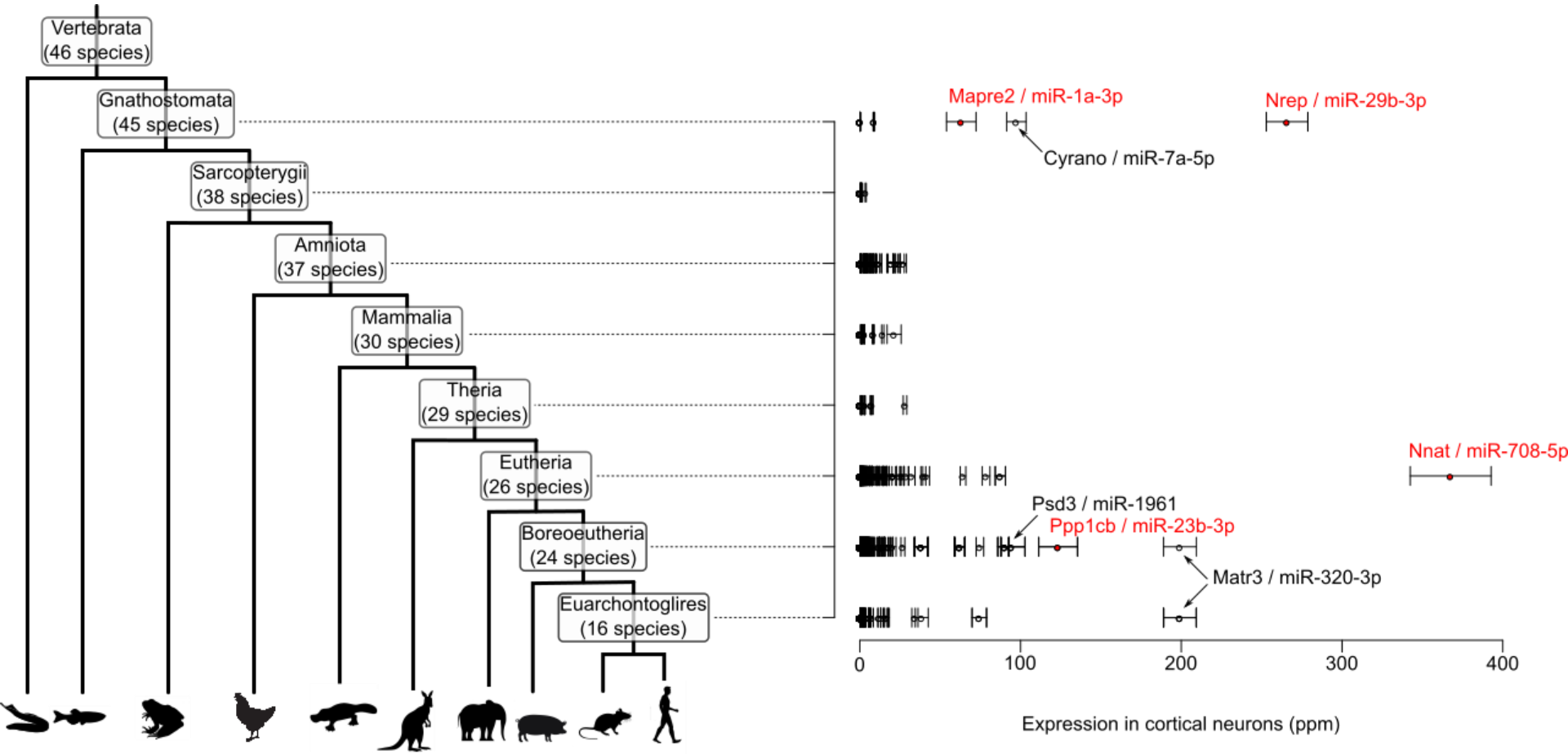
List of all putative TDMD-inducing sites in the human genome

For each record:
- position of the RNA site in human genome (chromosome, coordinates, strand)
- genomic annotation of the RNA site (coding exon, intron, intergenic region, UTR)
- paired microRNA name
- geometry of the pairing (extensive complementarity, central bulge or seedless)
- conservation (list of other vertebrates for which a TDMD pairing is also found for this site in their genomes)



De la Mata et al, 2015 (EMBO Rep)

Prediction of TDMD-inducer candidates in cortical neurons

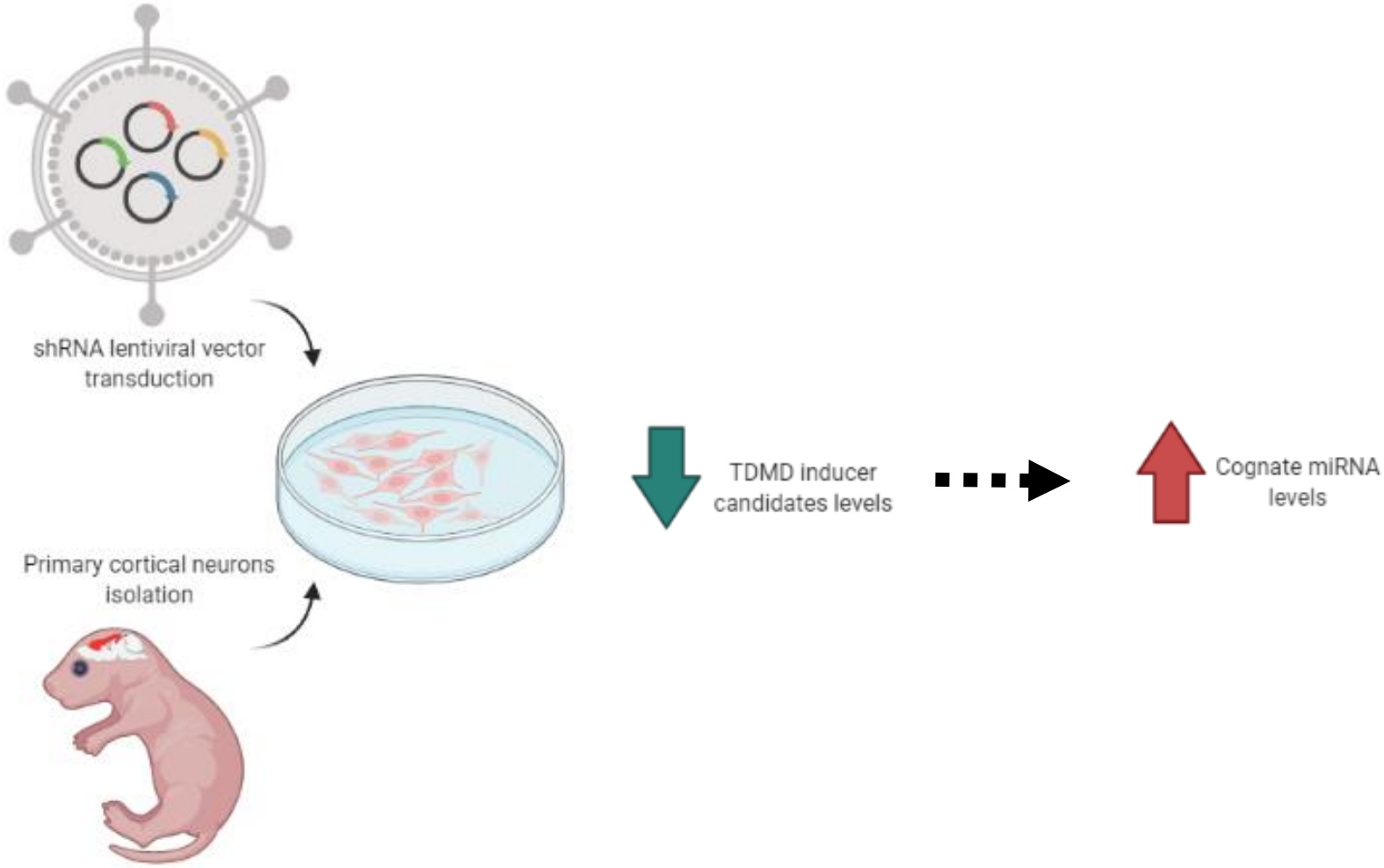


X-axis: Expression of TDMD site host RNA in cortical neurons (ppm)

Y-axis: Ranking of TDMD sites by clade specificity

(a site is specific to a phylogenetic clade when present in >50% of species in the clade and absent in every species outside the clade)

Experimental validation of TDMD inducer candidates in cortical neurons



To be continued…

Thank you for your attention

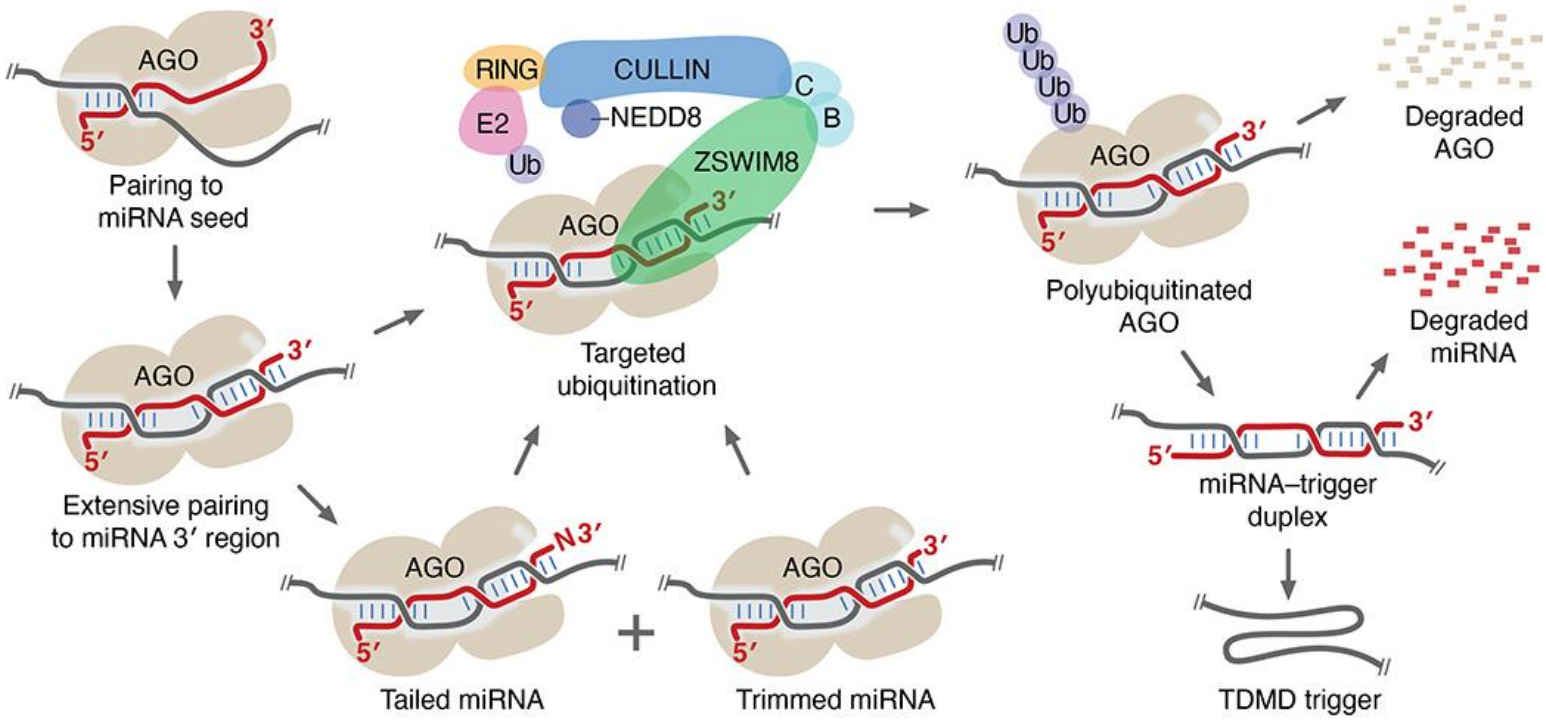
Acknowledgment

Seitz Lab
Hervé Seitz
Isabelle Busseau
Elisabeth Houbron
Germain Busto

Perroy Lab
(Institute of Functional Genomics, Montpellier)

Mail: sophie.mockly@igh.cnrs.fr

Suppl. Slide:



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Suppl. Slide:

Database miRBase
All mature miRNA sequences
Format fasta file

```
>cel-let-7-5p MIMAT0000001 Caenorhabditis elegans let-7-5p
UGAGGUAGUAGGUUGUAUAGUU
>cel-let-7-3p MIMAT0015091 Caenorhabditis elegans let-7-3p
CUAUGCAAUUUUCUACCUUACC
```

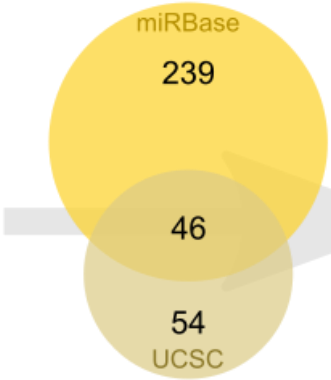
Database UCSC
Human vs 99 vertebrate genomes
Format multiple alignement file (1 per chromosome)

```
a score=1380.000000
s hg38.chrY                10849 20 + 57227415 TGCAAACACTTTGTACGAAA
s rheMac3.chrUn_JH290449     748 20 +    18974 TGCCAACATTTTGTAGACAA
i rheMac3.chrUn_JH290449 C 0 C 0
s colLiv1.KB375582        237180 19 -  1274627 TGCAAAC-CTTCATGCTGGA
i colLiv1.KB375582        N 0 C 0

a score=1184.000000
s hg38.chrY                11200 11 +  57227415 CTATGGCTTCT
s rheMac3.chrUn_JH290449    1099 11 +     18974 CTATGGGTTCT
i rheMac3.chrUn_JH290449 C 0 I 1354
s allMis1.JH732223        485913 11 +    622340 CCCTGGACATC
i allMis1.JH732223        I 1 C 0
```

block

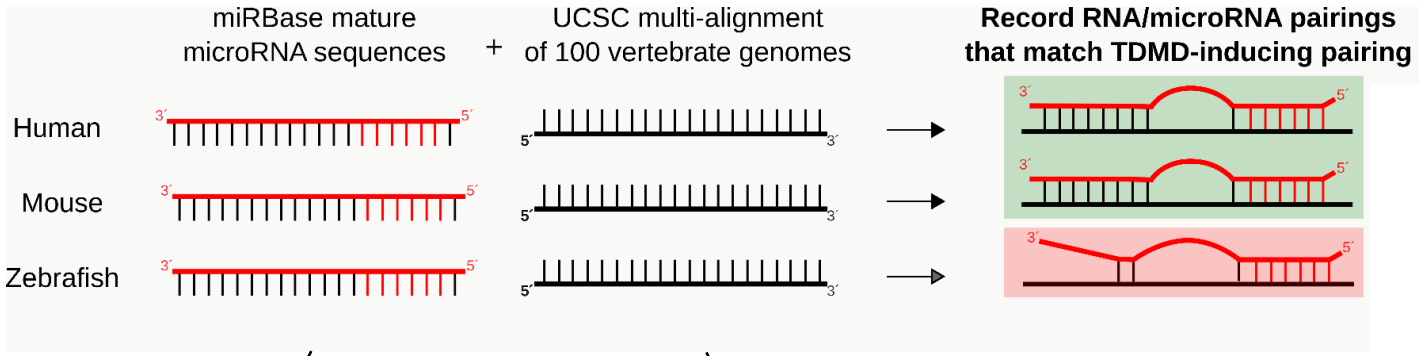
one aligned sequence



Index of common species names

#Latin_species	miRBase_prefix	UCSC_assembly
Homo sapiens	hsa	hg38
Callithrix jacchus	cja	calJac3
Mus musculus	mmu	mm10

1. Search of TDMD-inducing pairing patterns



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i colLiv1.KB375582        N 0 C 0

a score=1184.000000
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one aligned sequence

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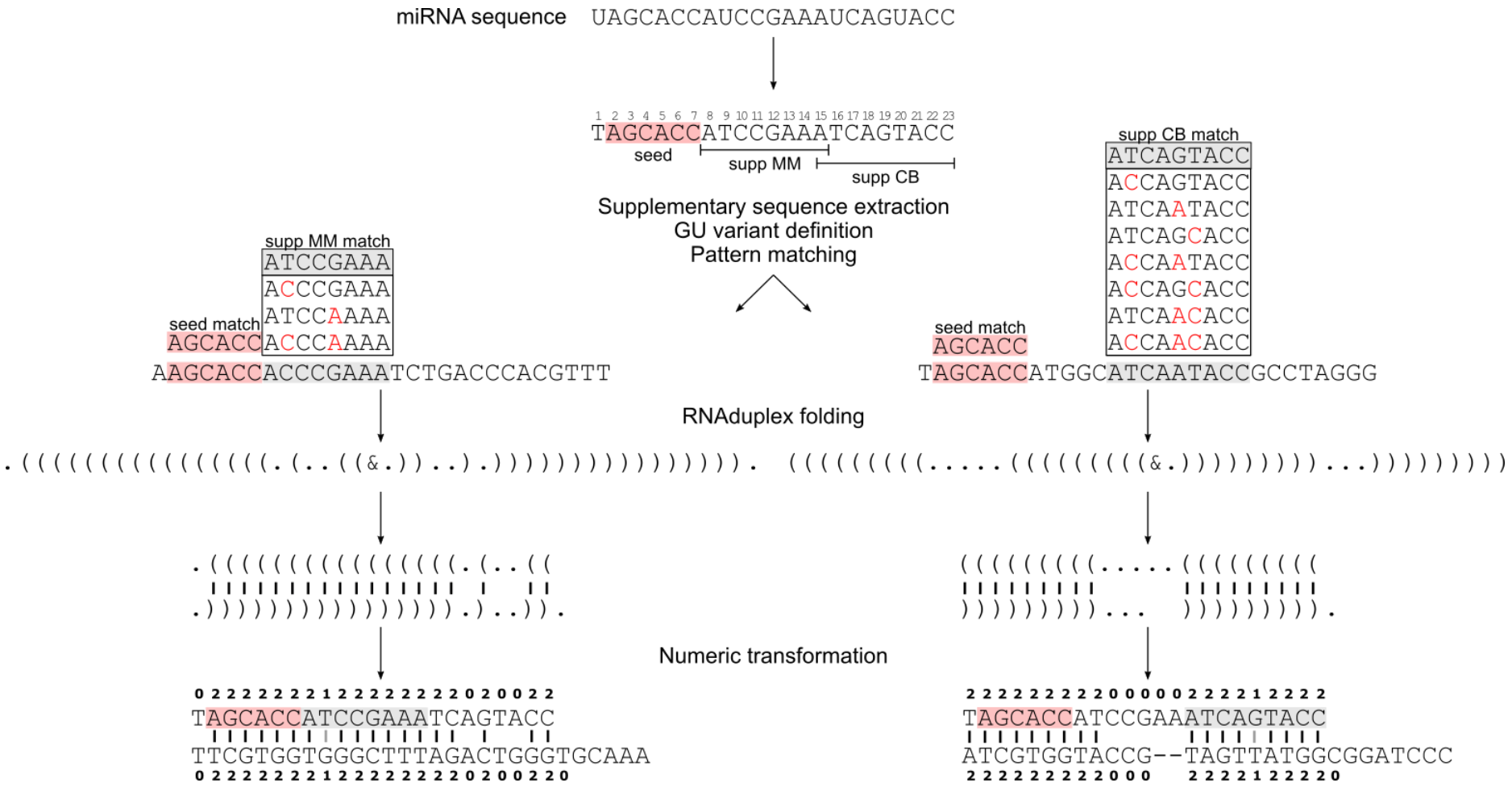

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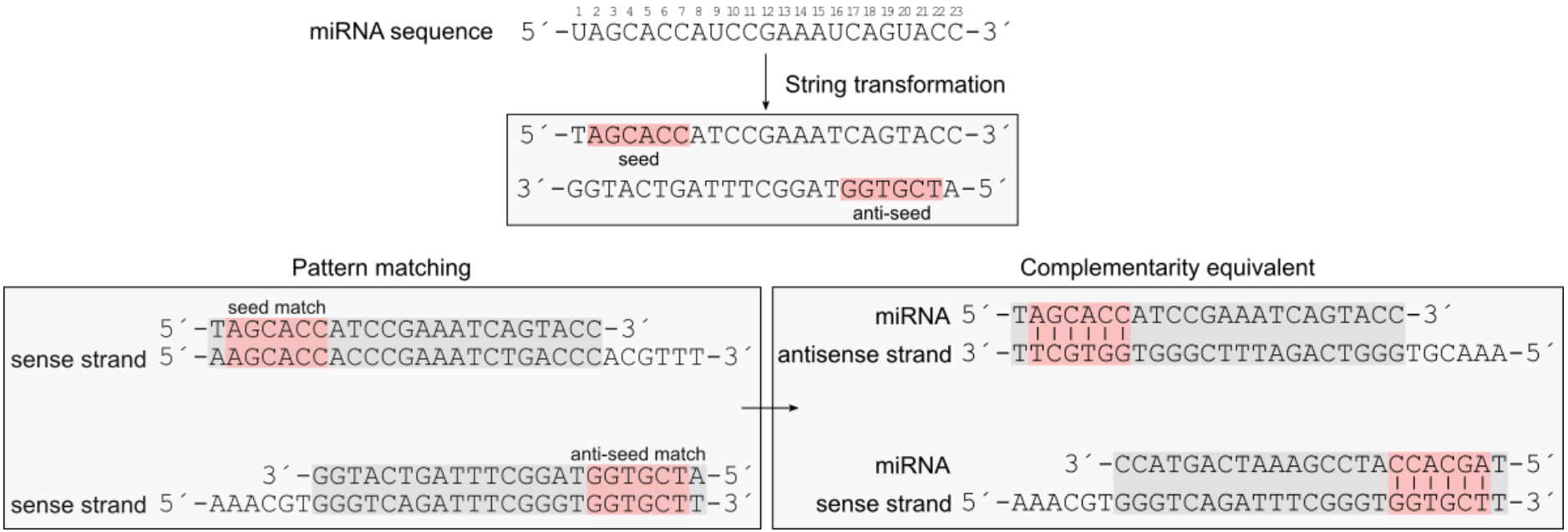
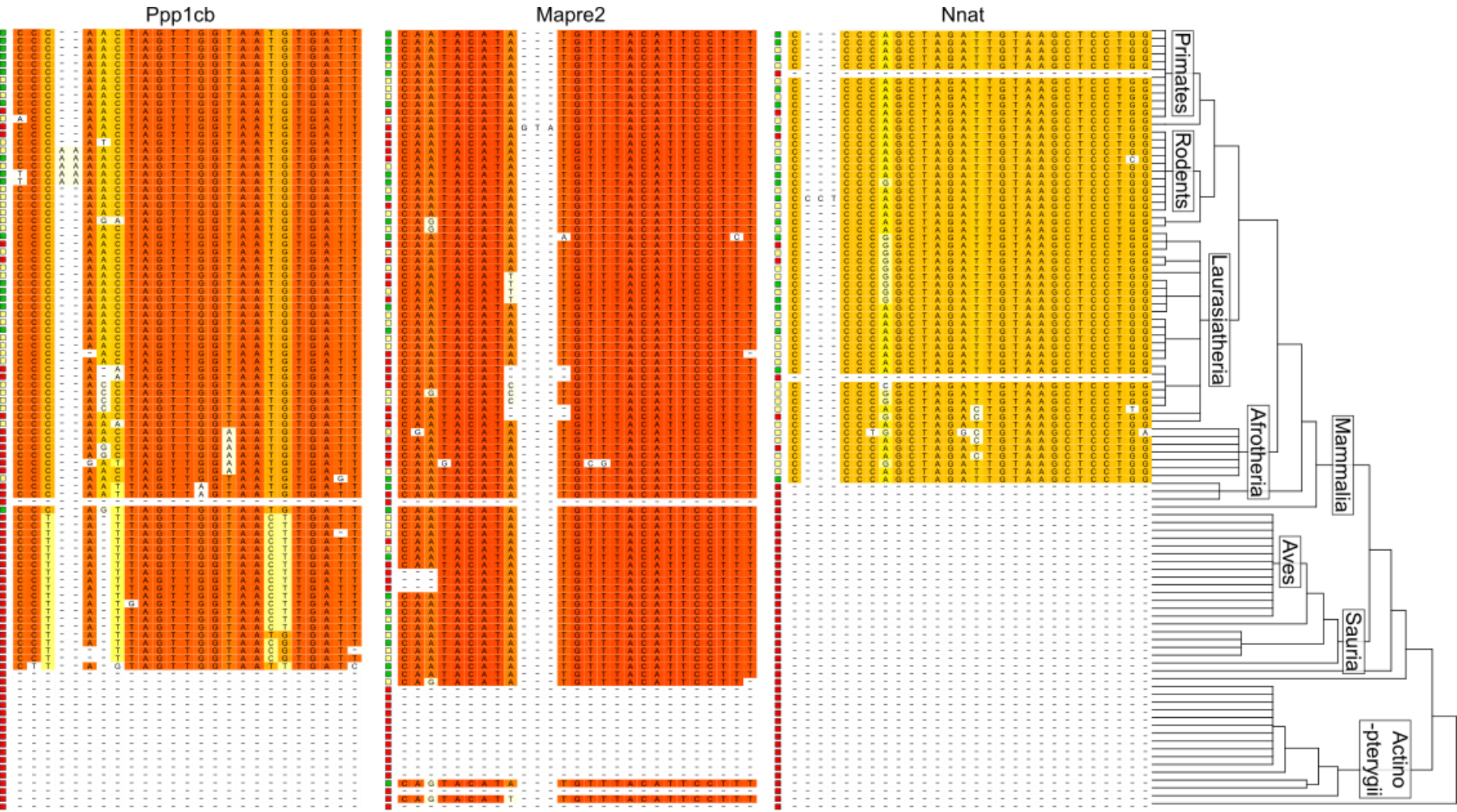


Prediction of TDMD-inducer candidates in cortical neurons



Conserved in:

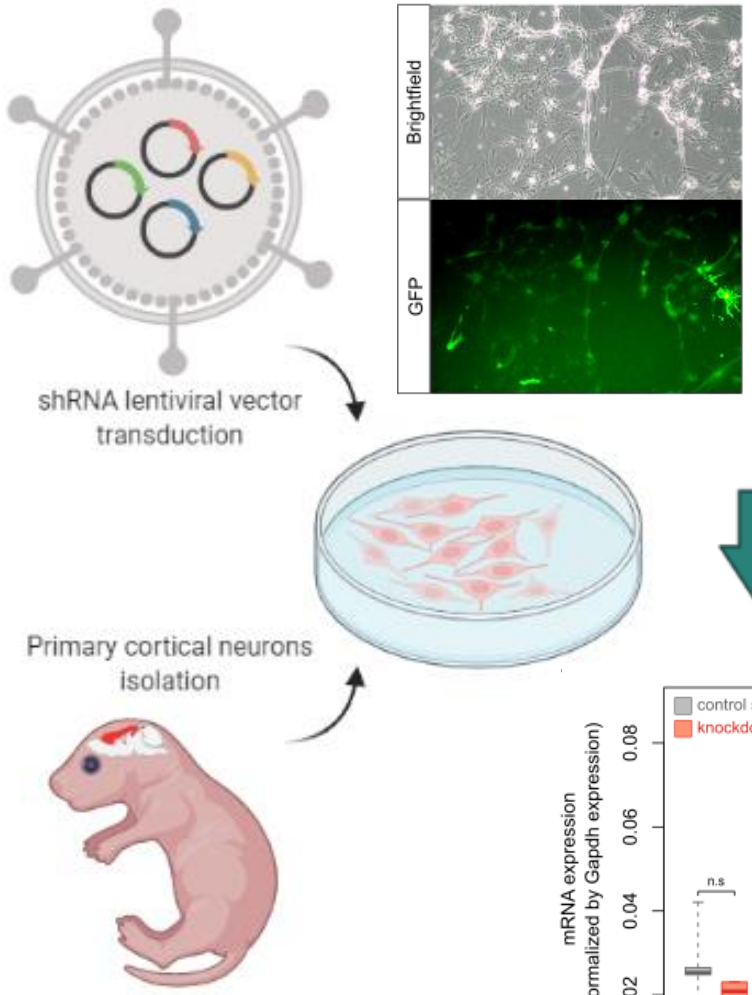
0% 50% 100%

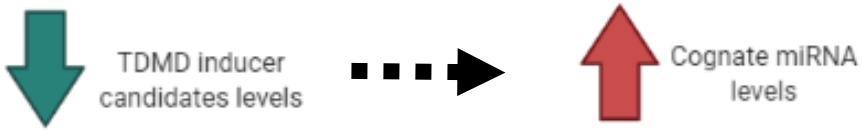
of the 100 aligned vertebrate species

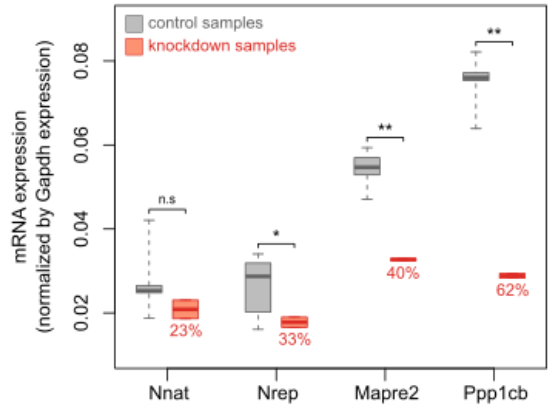
Existing TDMD-compatible miRNA in the species:

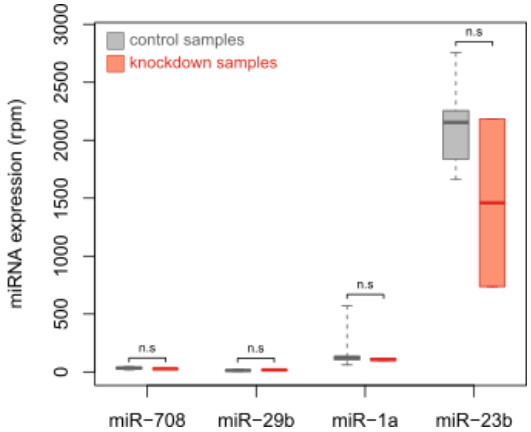
- one miRBase-annotated miRNA
- one candidate miRNA
- no miRNA

Experimental validation of TDMD inducer candidates in cortical neurons



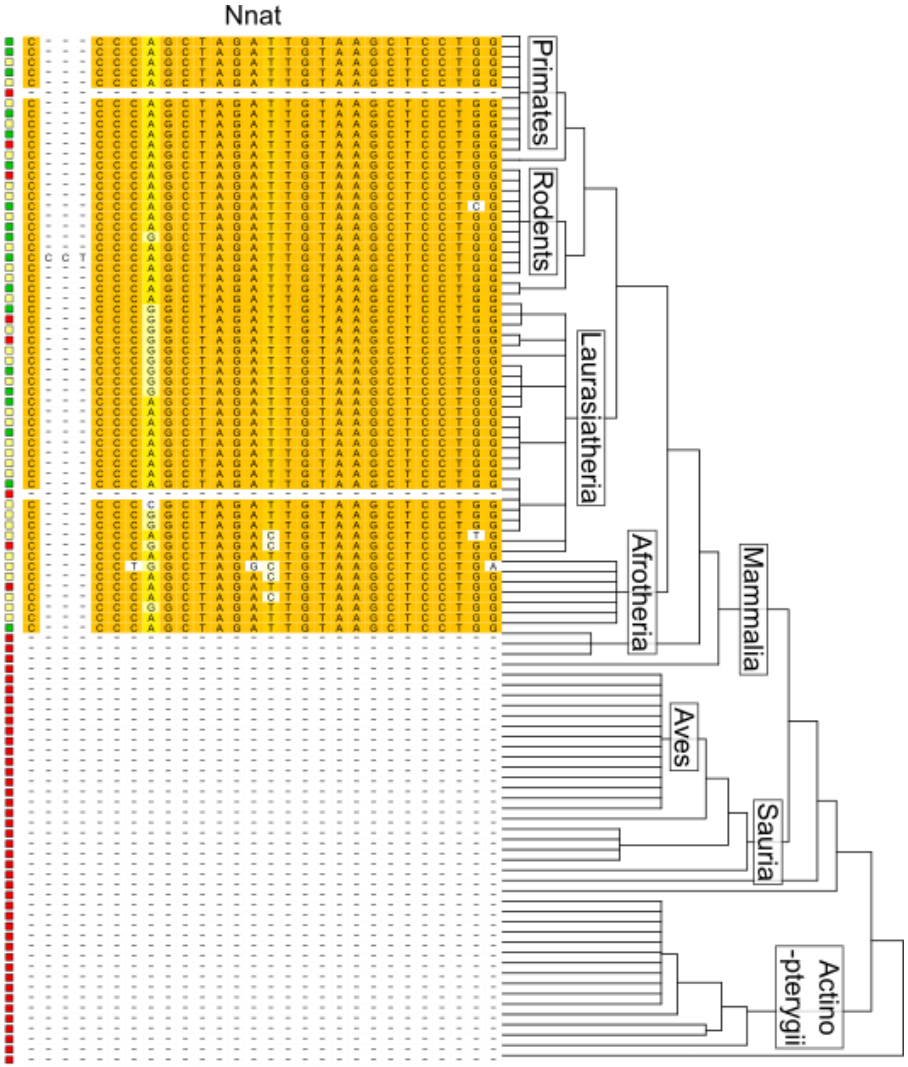






To be continued…

Experimental validation of AGO2-miR-708 cleavage activity



Conserved in:

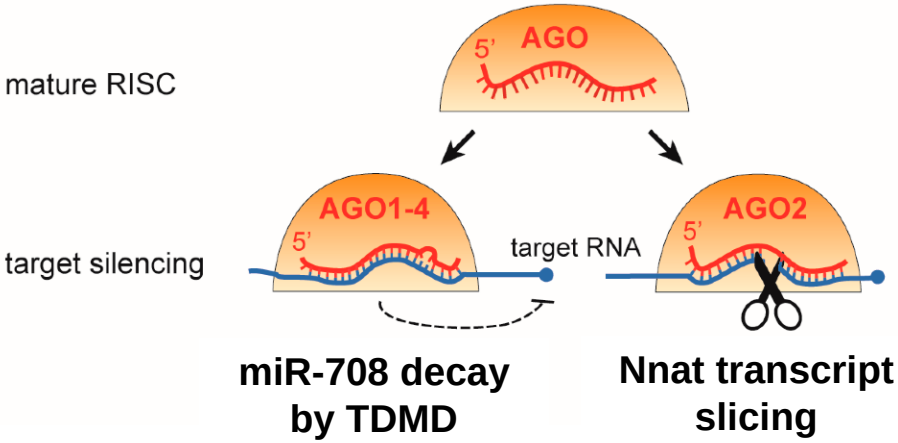
0% 50% 100%

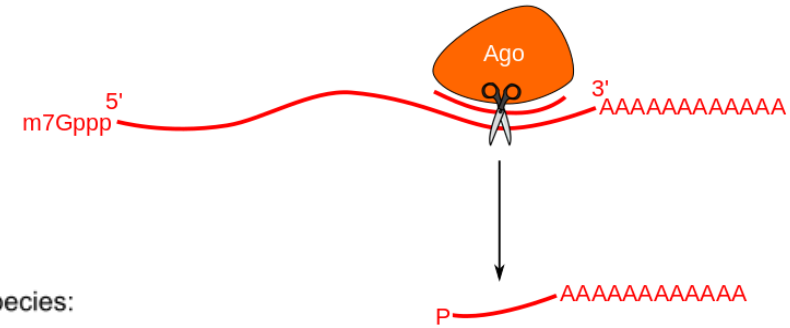
of the 100 aligned vertebrate species

Existing TDMD-compatible miRNA in the species:

- one miRBase-annotated miRNA
- one candidate miRNA
- no miRNA

```
hsa-miR-708-5p:GGGUCGAUCUAACAUUCGAGGAA
                |||||||||||||||||||||||X
hsa-Nnat:    5'CCCCAGCUAGAUUGUAAGCUCCUGG 3'
```





Identification of clived Nnat transcripts by RLM 5'-RACE
= RNA Ligase-Mediated-5' Rapid Amplification of cDNA Ends

Experimental validation of AGO2-miR-708 cleavage activity